Remarks

Claims 3, 57-73, and 76-78 are pending. Claim 57 has been amended. Claim 86 has been canceled. Claim 57 was amended to clarify any marker sequences that may be present following retrieval of the microorganisms from the particular environment.

Priority

(A) With regard to the issues raised by the examiner relating to the 09/201,945 and 08/637,759 priority applications, the applicant disagrees with the examiner's assertions. ***This application is a continuation of 08/201,945 and 08/637/759 – accordingly, the specification of the priority applications is identical to the specification of the present application.*** Each of 09/201,945 and 08/637,759, as well as the present application, satisfy the priority, written description, enablement and clarity requirements, as discussed in more detail below.

For example, see page 11, lines 10-12, where the specification states "it will be appreciated that although transposons are convenient for insertionally inactivating a gene, any other known method, or method developed in the future may be used." Furthermore, page 11, lines 12 to 17 discuss "insertion-duplication mutagenesis"; page 11, lines 19 to 26 refer to the use of DNA fragments or plasmids; and page 11, lines 28 to 30 refer to the use of Ty elements and ribosomal DNA in yeast. It is perfectly clear that the claimed methods are not limited to the use of transposons and transposon-like elements.

The Examiner is also referred to page 12, lines 3 and 4, wherein the purpose of introducing a marker sequence into the genome of the microorganism such that "each mutant contains a different marker sequence." This is exactly what is in claim 57, step (a):

"(a) providing a plurality of mutant microorganisms wherein 'each mutant contains a different marker sequence."

The specification, as indicated above, makes it plain that such mutants may be made using any suitable means, and not just through the use of transposons.

Step (d) requires the comparison of the unique tag sequences (i.e. the different marker sequences, if any) in surviving microorganisms with the tags of the plurality of mutant microorganisms introduced into the particular environment (i.e. those of step (a) of the method). It is those individual mutants having a reduced capacity to proliferate in the particular environment which are selected is described at page 4, lines 20 and 21, where it is noted that the method uses negative selection to identify microorganisms with a reduced capacity to proliferate in the environment. The present claims are properly based on subject matter present in the original applications. WO 96/17951, to which this application claims priority, clearly and explicitly teaches the subject matter presently claimed.

With regard to the **specification**, there is no need to amend the specification since the claims are properly based on subject matter present therein. Furthermore, a new **oath/declaration** is not required since the claims are properly supported by the original applications.

Rejection under 35 U.S.C. 112, first paragraph

Claims 3, 57-73, 76-78 and 86 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Any confusion over whether the "original" microorganisms are mutants is resolved by deleting claim 86. The preamble to claim 57 makes explicit what is implicit from the method steps, which is that the claimed method is used to identify a mutant microorganism with reduced adaptation to a particular environment.

It is not important to the operation of the method how the mutant microorganisms are generated provided that each mutant microorganism originally contains a different marker sequence which can be used to track the fate of the mutant microorganism in the particular environment. This is perfectly clear from the original application at page 12, lines 3 and 4, wherein "each mutant contains a different marker sequence."

The term "marker sequence" is self-defining in that it is a DNA sequence that marks the mutant (and each different mutant is marked with a different marker sequence in order to follow the fate of each mutant in the particular environment). Furthermore, the term "tag" is not used in the claims.

With regard to whether or not the claims link the marker sequence obtained in step (c) of claim 57 to that originally present in the microorganism, step (d) makes it clear that marker sequences of the microorganisms retrieved in step(c) are compared to the different marker sequences originally present in the microorganisms (i.e. those referred to in step (a)).

Step (d) of claim 57 makes it explicitly clear that the individual mutant is selected by comparing any marker sequences in the nucleic acid present in the retrieved microorganisms to the markers originally present. This is described in the original application. See, for example, page 11, where a number of different methods for creating a mutant and introducing a marker sequence are described. Furthermore, page 13, lines 17 and 18, note that transposons may be used "*or other DNA sequence.*" The specification does describe other approaches for providing microorganisms having unique markers for use in the claimed method. Moreover, these were well known to those skilled in the art as of the time the application was filed. It is well established that one need not describe in detail that which is known to those in the field. Here, the invention is the use, not the discovery of, markers such as transposans.

In the paragraph bridging pages 7 and 8 of the office action mailed on November 17, 2003, the examiner alleges that the specification describes no other means of "selecting by comparison" of microorganisms having a reduced ability to adapt to a particular environment than comparison on nucleic acid tags present within transposable elements from surviving microorganisms to those present in the microorganisms prior to their insertion into the particular environment. The examiner then effectively concludes that there is no written description of step

(d) of claim 57. However, steps (5) and (6) of claim 1 of WO 96/17951 (to which this application claims priority and which is identical to this application as originally filed) provides a clear basis for step (d) of present claim 57, particularly when this is coupled with the disclosure at page 4, lines 20-21, which refers to the use of "negative selection to identify microorganisms with reduced capacity to proliferate in the (particular) environment." Upon a reading of the present specification, there is no doubt that the skilled person is able to envision step (d) of claim 57.

The Examiner is referred to Hensel *et al.* (*Science* 269, 400-403, 1995) which describes the same method as described in the original patent application (this is not prior art, but was published shortly after the priority date, by the present inventors). This paper is a seminal paper in using marker sequences to mark (or "bar code") mutant strains of microorganisms and follow their fate in particular environments, and has been widely cited and readily implemented using a variety of methods of mutation, a variety of different environments and in each case by comparing any marker sequences in the nucleic acid present in the retrieved microorganisms with the different marker sequences in the plurality of mutant microorganism initially introduced into the particular environment.

The examiner is also referred to Shoemaker et al. (Nature Genetics, 1996), which describes quantitative phenotype analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy. In particular, the abstract notes that "*each deletion strain* [ie mutant] *is labelled with a unique 20-base tag sequence" and that the "tags serve as unique*

identifiers (molecular bar codes) that allow analysis of large numbers of deletion strains simultaneously through selective growth conditions". Reference 6 is Hensel *et al* (1995). Page 451, paragraph spanning columns 1 and 2 sets out the steps of molecular bar-coding which, as can be seen, is an implementation of the method of the PCT WO 96/17951 priority application.

See also Winzeler *et al* (1999) *Science* **285**, 901-906, describing the functional characterisation of the *Saccharomyces cerevisiae* genome "*by marking each yeast gene with a molecular "bar code" that allows the phenotypes of the mutant strains to be assayed in parallel*" (see page ..., column 2, lines 5 to 7). Reference 7 is Hensel *et al* (1995). In this case various "particular environments" were used such as "minimal medium" or "rich medium" and the strains which exhibit a growth defect are identified by comparing the "bar codes" (ie marker sequences) with an array of probes representative of all strains introduced into the environment (see legend to Figure 2).

WO 01/53532 relates to bar-coded synthetic lethal screening to identify drug targets and Hensel *et al* (1995) is cited on page 4, lines 1 and 2.

"Bar-coded" mutants are used which are, in this case, introduced into an environment containing particular drugs and the question is asked whether particular mutants survive in the presence of drugs (see page 2, line 34 to page 3, line 10). The method makes use of double mutants (to find the effect of a secondary mutation on a primary mutation) but the principle is the same which is illustrated on page 3, lines 2 to 10 which notes:

"*Because each mutated clone is tagged (barcoded), the relative abundance of each clone can be easily determined by assaying for each of the tags. This may be done, for example, by hybridizing DNA obtained from the culture to a DNA microarray consisting of DNA molecules complementary to each tag. Missing tags represent those clones that harbor a "synthetic lethal" secondary mutation, i.e., a mutation that interacts with a primary mutation resulting in decreased rate of growth of the cell harboring both the primary and the secondary mutation. Because the library of mutants is tagged and characterized, identification of under-represented tags in the library after introduction of the primary mutation is tantamount to identifying a gene product which, if knocked out, causes a decreased growth rate when combined with the primary mutation.*"

Thus, WO 01/53532 readily implements the claimed method.

Lastly, the examiner is referred to Lum *et al* (*Cell* 116, 121-137, 2004). Although this paper does not cite Hensel *et al* (1995) it plainly implements the claimed method in the circumstance where "tagged" or "bar-coded" *S. cerevisiae* mutants are introduced into a particular environment (ie growth medium containing a drug). This is evident from, for example, Figure 1B which notes that the "*tag is missing due to sensitivity of strain to drug*" and in column 2 on page 122 where it is noted that "[d]*eletion strains that were sensitive to a given compound were out competed by thousands of unaffected strains in the pool*".

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 3, 57-73, 76-78 and 86 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The term "any" is perfectly clear since it plainly means any marker sequence which are present following retrieved of the microorganisms from the particular environment. However, to expedite prosecution, the applicant has amended claim 57 to recite "... *by comparing marker sequences, if any, in the nucleic acid present ...*".

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 3, 57-73, 76-78 and 86 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

It is perfectly clear from the above that not only is the written description requirement satisfied but also the enablement requirement. In particular, the evidence is that the skilled person can (and has) readily made and used the claimed invention as demonstrated by the post-filing references discussed below, Winzeler *et al.* (*Science* **285**, 901-906, 1999); Lum *et al.*, (*Cell* 116, 121-137, 2004); Shoemaker *et al.* (*Nature Genetics*, 14, pgs. 450-456, 1996); and WO 01/53532.

Turning to the so-called *Wands* factors referred to by the examiner.

Nature of the invention

We agree that the nature of the invention is complex, but on the other hand the level of skill of the skilled person in this art is high. The examiner states that in order to practice the invention, there needs to be some correlation between the marker sequences present in the microorganisms that survive compared to those initially introduced into the environment.

We agree and this is precisely what is set out in step (d) of the method where an individual mutant having a reduced capacity to proliferate in the particular environment by comparing [ie correlating] any marker sequences in the nucleic acid present in the retrieved microorganisms with the different marker sequences in the mutant microorganisms initially introduced.

Breadth of claims

The embodiments claimed are described in the specification as discussed above.

Guidance of the specification/The existence of working examples

This has already been dealt with above since the description does describe different mutants containing different marker sequences made other than by using transposons, and methods of identifying mutant microorganism which are not adapted to survive in the particular environment (e.g. by any negative selection criterion (for example, see page 4, lines 20 and 21)).

There are clear working examples of the method claimed and the skilled person has no practical difficulty in carrying out the invention claimed (as evidenced by the post-filed references described above).

State of the art

The examiner is correct that the *prior art* does not anticipate the claimed subject matter. However, the *state of the art* was generally highly developed and the skilled person had no difficulty in carrying out the methods using different microorganisms, different types of mutations, different markers sequences, different "particular environments" and different ways of identifying those mutants with a reduced adaptation to a particular environment but in each case by comparing any marker sequences in the nucleic acid present in the retrieved mutant microorganisms with that in the plurality of microorganisms initially introduced into the particular environment.

Predictability of the art/Amount of experimentation required

The applicant disagrees that it would have been unpredictable to attempt the claimed method. Making mutations in a variety of different microorganisms was well established, as was the ability to introduce particular DNA sequences (i.e. marker sequences) into the genome of a microorganism. Similarly, introducing a plurality of such mutant microorganisms into a particular environment, allowing those able to grow in the environment to do so, and retrieving microorganisms from the environment could readily be done without undue experiment and with predictable results.

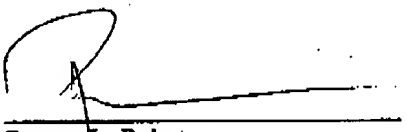
Similarly, comparing any marker sequences present in the nucleic acid present in the retrieved microorganisms with the different marker sequences in the plurality of microorganisms

initially introduced into the environment could readily be done without undue experiment and with predictable results.

It is important for the examiner to appreciate the very important contribution to the art made by the inventors, as defined by the claims. Implementation of this method, in a variety of ways, across the scope of the claims, is readily achieved by the skilled person without undue experimentation and in a predictable way as is evidenced by the post-filed references referred to below, all of which acknowledge the contribution made by the inventor.

Allowance of claims 3, 57-73, and 76-78 is respectfully solicited.

Respectfully submitted,



Patrea L. Pabst
Reg. No. 31,284

Date: February 17, 2004

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

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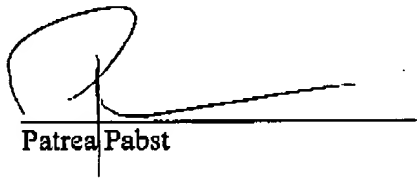
AMENDMENT AND RESPONSE TO OFFICE ACTION

Certificate of Facsimile Transmission

I hereby certify that this Amendment and Response to Office Action, and any documents referred to as attached therein are being facsimile transmitted on this date, February 17, 2004, to the Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450.



Patrea Pabst

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